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E. R. Pfefferkorn
Dartmouth College

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Interferon γ blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan

(tryptophan/kynurenine/*N*-formylkynurenine)

E. R. PFEFFERKORN

Department of Microbiology, Dartmouth Medical School, Hanover, NH 03756

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ABSTRACT **Treatment of human fibroblasts with human recombinant γ interferon blocked the growth of *Toxoplasma gondii*, an obligate intracellular protozoan parasite. Growth of the parasite was measured by a plaque assay 7 days after infection or by the incorporation of [^{3}H]uracil 1 or 2 days after infection. The antitoxoplasma activity induced in the host cells by γ interferon was strongly dependent upon the tryptophan concentration of the medium. Progressively higher minimal inhibitory concentrations of γ interferon were observed as the tryptophan concentration in the culture medium was increased. Treatment with γ interferon did not make the cells impermeable to tryptophan. The kinetics of [^{3}H]tryptophan uptake into the acid-soluble pools of control and γ interferon-treated cultures were identical during the first 48 sec. Thereafter uptake of [^{3}H]tryptophan into the acid-soluble pool of control fibroblasts reached the expected plateau after 96 sec. In contrast, uptake of [^{3}H]tryptophan continued for at least 12 min in the γ interferon-treated cultures. At that time, the acid-soluble pool of the γ interferon-treated cultures contained 8 times the radioactivity of the control cultures. This continued accumulation was the result of rapid intracellular degradation of [^{3}H]tryptophan into kynurenine and *N*-formylkynurenine that leaked slowly from the cells. These two metabolites were also recovered from the medium of cultures treated for 1 or 2 days with γ interferon. Human recombinant α and β interferons, which have no antitoxoplasma activity, did not induce any detectable degradation of tryptophan. Several hypotheses are presented to explain how the intracellular degradation of tryptophan induced by γ interferon could restrict the growth of an obligate intracellular parasite.**

Interferons are currently assigned to three classes on the basis of the cells that produce them (reviewed in ref. 1); α and β interferons (IFN-α and IFN-β) are produced by leukocytes and fibroblasts, respectively, in response to viral infection, whereas γ interferon (IFN-γ) is produced by T-lymphocytes when stimulated by mitogens or by antigens to which they had previously been sensitized. Each of these interferons was originally identified through its inhibition of viral growth. In addition to their well-documented antiviral activities, crude interferons also occasionally have been observed to suppress the growth of other nonviral intracellular infectious agents (reviewed in ref. 2). In studying the antiviral-like action of interferon against *Toxoplasma gondii*, an obligate intracellular protozoan parasite, it is important that macrophages not be used as the host cells. Activated macrophages are known to kill intracellular *T. gondii* (3), and macrophage-activating factor might be present in crude interferon preparations. Indeed, macrophage-activating factor and IFN-γ may well be the same substance. Several laboratories have reported that interferon blocked the growth of *T. gondii* in cultured fibroblast or epithelial cells (4–6). Shirahata and Shimizu (6) have presented evidence that IFN-γ is the active substance in their studies of the inhibition of *T. gondii*. Experiments done in this laboratory showed that human recombinant IFN-α and IFN-β had no effect on *T. gondii*. However, treatment of human fibroblasts with 8 to 16 units of human recombinant IFN-γ per ml blocked the growth of *T. gondii*. This IFN-γ inhibited vesicular stomatitis virus at 4 units/ml with the same line of human fibroblast as host cells (unpublished data).

The biochemical basis of the antiviral state induced by the various interferons is now reasonably well understood (reviewed in ref. 7). In reviewing the action of interferon on nonviral agents, Vilček and Jahiel (2) suggested 13 yr ago that inhibitory mechanisms different from those that block viral growth were likely to be involved. This report presents evidence for an antitoxoplasma mechanism induced by IFN-γ that is totally different from the well-studied antiviral state induced by interferons.

MATERIALS AND METHODS

Host Cells and Parasites. Human fibroblasts were grown in Eagle's (8) minimal essential medium supplemented with antibiotics and 10% fetal bovine serum. The serum concentration was reduced to 3% for infection or for treatment with interferon. Medium without tryptophan was prepared from a kit supplied by GIBCO and supplemented with dialyzed fetal bovine serum. Cloned *T. gondii* of the RH strain were maintained by serial passage in human fibroblast cultures and assayed by plaque formation, with the results reported as plaque-forming units as described (9).

Interferons and Their Titration. Human recombinant IFN-α, IFN-β, and IFN-γ were supplied by Genentech (San Francisco, CA) (10). They were stored at 4°C at 10^6 units/ml in 0.5 M NaCl/20 mM Tris, pH 8/1% 2-mercaptoethanol. All interferon concentrations reported here are based on assays done at Genentech with HeLa cells and vesicular stomatitis virus. Titrations done in this laboratory with human fibroblasts and vesicular stomatitis virus found these interferons slightly less potent. The lowest concentrations that suppressed the growth of vesicular stomatitis virus in human fibroblasts were 2, 2, and 4 units/ml for the IFN-α, IFN-β, and IFN-γ, respectively. Plaque reduction assays (unpublished data) of the activity of IFN-γ against *T. gondii* were carried out in parallel with every experiment reported here and always showed complete inhibition of plaque formation at 8 to 16 units/ml. The extent of the inhibition of the growth of *T. gondii* was measured 1 and 2 days after infection by the incorporation of [^{3}H]uracil. This precursor is incorporated by *T. gondii* but not by the host cell because only the parasite contains significant quantities of uracil phosphoribosyltransferase (EC 2.4.2.9) (11). Infected human fibroblast monolayer cultures in trays that contain 24 wells 1.6 cm in diameter were labeled by the addition of 5 μCi (1 Ci = 37 GBq) of [^{3}H]uracil without changing the medium. Two hours later the acid-precipitable radioactivity was measured. Because *T. gondii* has a generation time of 7–8 hr, incorpo-

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ration of [^{3}H]uracil during a brief pulse 1 or 2 days after infection is a valid measure of parasite growth. In parallel experiments, the antiparasitic activity of IFN-γ measured by this radiometric assay of *T. gondii* growth was identical to the activity obtained by measuring the inhibition of parasite growth in a plaque assay (unpublished data).

Acid-Soluble Pools. The uptake of [^{3}H]tryptophan into the acid-soluble pool of human fibroblasts was measured by using confluent cultures grown on round glass coverslips 1.2 cm in diameter. These coverslips were dipped into Hanks' (12) balanced salt solution to wash away the existing medium and then incubated at 37°C for 6 sec or longer in fresh medium that contained [^{3}H]tryptophan (75 μCi/ml). The exposure to this radioactive medium was ended by four successive dips in ice-cold phosphate-buffered saline. The acid-soluble pool was then extracted with cold 0.3 M trichloroacetic acid for 1 hr. The acid extract was centrifuged at 12,000 $\times$ *g* for 30 sec, and the radioactivity of the supernatant fluid was measured.

Chromatography and Electrophoresis. Tryptophan (R_f = 0.65) was separated from a mixture of *N*-formylkynurenine and kynurenine (R_f = 0.85) by ascending chromatography on Whatman 3MM paper in 0.1 M HCl. All three substances were resolved by a two-dimensional separation that used thin-layer cellulose ascending chromatography at pH 1.85 in a solution that contained 0.67 M acetic acid and 0.5 M formic acid, followed by electrophoresis (50 V/cm) in the same solution without drying the thin layer. Spots were located by fluorescence under long UV illumination, by the ninhydrin reaction, or by autoradiography using Kodak XPR-1 x-ray film.

Charcoal Adsorption. The aromatic compounds in the medium of cultures treated with interferon were concentrated and desalted by adsorption onto and elution from charcoal. Medium that had been prepared without phenol red was shaken with charcoal (1 mg/ml) for 30 min. The charcoal was collected by centrifugation and washed three times with water. The adsorbed substances were eluted in ammonium hydroxide/ethanol, 8:5 (vol/vol). The eluate was passed through a 0.22-μm filter to remove residual charcoal and then evaporated dry under a stream of N_2.

Chemicals and Radiochemicals. L-Tryptophan, L-kynurenine, and L-*N*-formylkynurenine were purchased from Calbiochem. L-[G-^{3}H]Tryptophan (2.8 Ci/mmol) and L-[*methylene*-^{14}C]tryptophan (58 mCi/mmol) were purchased from Amersham.

RESULTS

The minimal concentration of IFN-γ that blocked the growth of *T. gondii* as measured by the specific incorporation of [^{3}H]uracil was consistently 2-fold higher in Dulbecco's (13) modified minimal essential medium than it was in Eagle's (8) original minimal essential medium. Individual tests of each of the 27 quantitative and qualitative differences between these two media showed that the critical ingredient was tryptophan, which is supplied at 10 μg/ml in Eagle's medium and 16 μg/ml in Dulbecco's medium. A systematic analysis of the dependence of the IFN-γ activity upon the tryptophan concentration in the medium is shown in Fig. 1. The data from infected cultures that were not treated with IFN-γ showed that the growth of *T. gondii* was independent of the tryptophan concentration in the medium. One day after infection, the same incorporation of [^{3}H]uracil was observed in cultures with tryptophan concentrations that ranged from 0 to 30 μg/ml. Normal short-term growth of *T. gondii* within cells incubated in medium lacking tryptophan does not prove that the parasite can synthesize this amino acid. *T. gondii* may well have grown at the expense of tryptophan in the acid-soluble pool of the host cell, which would be replenished by protein turnover in the absence of this essential amino acid in the medium.

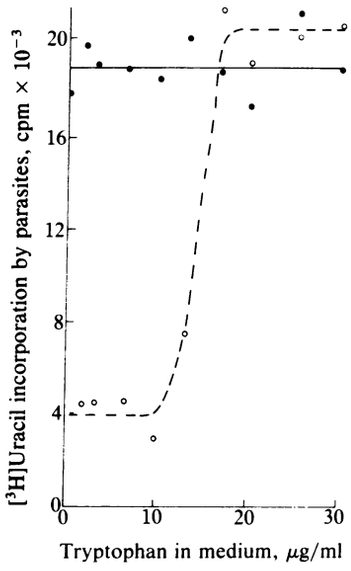

FIG. 1. The effect of tryptophan concentration on the inhibition of the growth of *T. gondii* in cells treated with IFN-γ. Quadruplicate cultures in 24-well trays were treated with control medium or with medium that contained 16 units of IFN-γ per ml for 24 hr. The cultures were then infected with 3 $\times$ 10^5 plaque-forming units. Twenty-four hours after infection, each culture was labeled with 5 μCi of [^{3}H]uracil, and the acid-precipitable radioactivity was measured 2 hr later. ●, Control cultures; ○, cultures treated with IFN-γ.

Fig. 1 shows that the concentration of tryptophan in the medium markedly affected the antitoxoplasma activity of IFN-γ at 16 units/ml, which was the minimal inhibitory concentration in the titration carried out in Eagle's medium. Doubling the 10 μg of tryptophan found per ml of this medium completely blocked the effect of the IFN-γ. This observation was confirmed and extended by titrating the antitoxoplasma activity of IFN-γ in media prepared with four different tryptophan concentrations. Fig. 2 shows that these

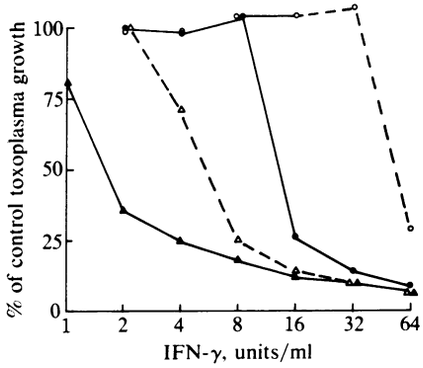

FIG. 2. Titration of the antitoxoplasma activity of IFN-γ in media with four tryptophan concentrations. Quadruplicate cultures in 24-well trays were treated with 2-fold dilutions of IFN-γ in four media that differed only in tryptophan content. Twenty-four hours later all cultures were infected with 3 $\times$ 10^4 plaque-forming units; 48 hr after infection, all cultures were labeled with 5 μCi of [^{3}H]uracil, and the incorporation into acid-precipitable material was measured 2 hr later. The results are expressed as a percentage of the incorporation observed in control cultures that were not treated with interferon. The growth of *T. gondii* in these cultures was not affected by the tryptophan content of the media (see Fig. 1). The concentrations of tryptophan in the media were 0 μg/ml (▲), 2 μg/ml (△), 10 μg/ml (●), and 50 μg/ml (○).

titrations yielded four roughly parallel curves with progressively more IFN-γ required to inhibit the parasite as the tryptophan concentration in the medium was increased.

The observed interdependence of the tryptophan concentration in the medium and the antitoxoplasma activity induced by IFN-γ in human fibroblasts suggested two models. In the first, interferon induces the host cell to degrade tryptophan. In the second model, interferon induces a defect in tryptophan transport by host-cell plasma membrane. If *T. gondii*, like its host cell, requires tryptophan for growth, markedly reduced transport of this amino acid by the plasma membrane of the host cell could result in starvation of the parasite. Such starvation might even preferentially affect the parasite because it grows within a cytoplasmic vacuole—the parasitophorous vacuole—that is thought to be derived, at least in part, from the plasma membrane of the host cell (14). If the vacuolar membrane shared the postulated permeability defect of the plasma membrane, tryptophan in the medium would have to pass this barrier twice before it could be available to the parasite.

The model of impaired tryptophan transport induced by IFN-γ was tested by comparing the uptake of radioactive tryptophan into the acid-soluble pools of control and treated cultures. The acid-soluble pool of the control cultures was labeled by [^{3}H]tryptophan with the expected kinetics in which saturation was achieved in about 96 sec (Fig. 3). The cultures that had been treated with 64 units of IFN-γ per ml for 1 day before measurement of [^{3}H]tryptophan uptake were identical to the controls during the first 48 sec but thereafter showed a continued increase in the amount of radioactivity derived from [^{3}H]tryptophan in their acid-soluble pools. Thus, the model in which IFN-γ induces a defect in tryptophan transport is clearly excluded. However, the increased accumulation of radioactivity in the acid-soluble pool of cultures treated with IFN-γ was a reasonable consequence of the alternative model in which IFN-γ induces the degradation of tryptophan. Degradation of tryptophan would upset the equilibrium between external and internal [^{3}H]tryptophan that was established within 96 sec in the control cultures. Rapid intracellular degradation of tryptophan would result in continued influx of this amino acid from the medium. If the degradation products escaped relatively slowly from the cells, radioactivity derived from [^{3}H]tryptophan would accumulate in the acid-soluble pool in the manner shown in Fig. 3.

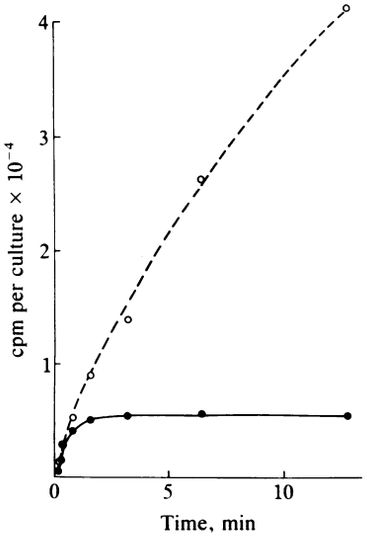

FIG. 3. The effect of treatment with IFN-γ on the labeling of the acid-soluble pool of human fibroblasts with [^{3}H]tryptophan. Confluent cultures on coverslips of 1.2-cm diameter were treated with 64 units of IFN-γ per ml or with control medium for 24 hr. The coverslip cultures were then dipped briefly into medium that contained 75 μCi of [^{3}H]tryptophan per ml, rinsed quickly, and extracted with cold trichloroacetic acid. Each point is the average of triplicate determinations. ○, Cultures treated with IFN-γ; ●, control cultures.

The tryptophan metabolites that accumulate within cells that were treated with IFN-γ do ultimately leak into the medium. When confluent human fibroblasts cultures were treated for 3 days with 64 units of IFN-γ per ml of medium that contained [^{14}C]tryptophan, the cpm/ml of the medium remained approximately the same as measured in daily samples. However, the percentage of the radioactivity that cochromatographed with authentic tryptophan decreased progressively from >95% in control cultures to <20% in cultures treated with IFN-γ for 2 days (Fig. 4). In this latter sample, most of the radioactivity in the medium migrated more rapidly than did tryptophan when analyzed by ascending paper chromatography in 0.1 M HCl. Cells treated with 16 units of IFN-γ per ml also degraded the tryptophan in their medium but at a slower rate. In contrast, cultures treated with 1,024 units of human recombinant IFN-α or IFN-β per ml showed no degradation of [^{14}C]tryptophan (data not shown). These interferons do not block the growth of *T. gondii* (unpublished data).

The first clue as to the identity of the metabolite(s) produced from tryptophan by cells treated with IFN-γ was the observation that the more rapidly migrating material (Fig. 4) fluoresced when exposed to long UV light. No such rapidly migrating fluorescent spot was seen on the chromatogram of control medium. To simplify their identification, the fluorescent tryptophan metabolites were prepared in a highly radioactive form. Two uninfected cultures were treated with 64 units of IFN-γ per ml of medium that contained [^{14}C]tryptophan at 10 μCi/ml. The medium from these cultures was harvested after 1 and 2 days of incubation, while control medium was harvested from a third culture after 2 days of incubation. Most of the tryptophan from the medium of the culture treated for 2 days with IFN-γ at 64 units/ml was converted to other radioactive metabolites (Fig. 4).

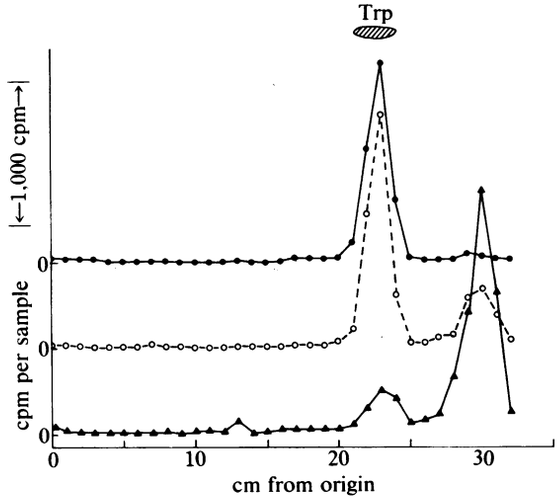

FIG. 4. Chromatographic demonstration of the destruction of tryptophan in the medium of uninfected human fibroblasts treated with IFN-γ. Confluent cultures were treated with medium that contained 64 units of IFN-γ per ml or with control medium in the presence of 5 μCi of [^{14}C]tryptophan per ml. Samples of centrifuged medium were supplemented with 2.5 mg of tryptophan per ml and analyzed by ascending paper chromatography in 0.1 M HCl. The tryptophan spots (Trp) were located through their fluorescence. The paper chromatographs were cut in 1-cm strips, and the radioactivity of each strip was determined. ●, Control cultures after 2 days of incubation; ○, IFN-γ-treated cultures after 1 day of incubation; ▲, IFN-γ-treated culture after 2 days of incubation.

Examination of various fluorescent degradation products of tryptophan showed that most of the radioactivity was associated with two substances, kynurenine and *N*-formylkynurenine. Part of the evidence for this identification is shown in Fig. 5, which presents autoradiographs of a two-dimensional thin-layer chromatographic/electrophoretic procedure that completely resolved tryptophan, kynurenine, and *N*-formylkynurenine. These three known compounds were added to the medium samples just before analysis and were subsequently located by the ninhydrin reaction. All of the radioactivity in the medium of cultures incubated for 2 days without interferon migrated with tryptophan (Fig. 5*A*). However, after 1 day of incubation with cells treated with IFN-γ, some of the radioactivity supplied as tryptophan migrated with kynurenine and *N*-formylkynurenine (Fig. 5*B*). Most of the radioactivity from the medium of cultures treated with IFN-γ for 2 days, migrated with kynurenine, with lesser amounts associated with *N*-formylkynurenine and tryptophan. Fig. 5*C* also shows evidence of several additional minor radioactive metabolites of tryptophan that have not yet been identified. The identification of the principal radioactive metabolite of tryptophan as kynurenine was confirmed by observing cochromatography of the authentic compound, located by ninhydrin, and the ^{14}C-labeled metabolite, located by autoradiography, in each of 10 different chromatographic solvents. This identification was further confirmed by fluorescence spectroscopy with unlabeled material that had been isolated from the medium of interferon-treated cultures by adsorption onto and elution from charcoal and then purified by two-dimensional chromatography/electrophoresis. The principal tryptophan metabolite and authentic kynurenine had similar fluorescence emission spectra with the same maxima.

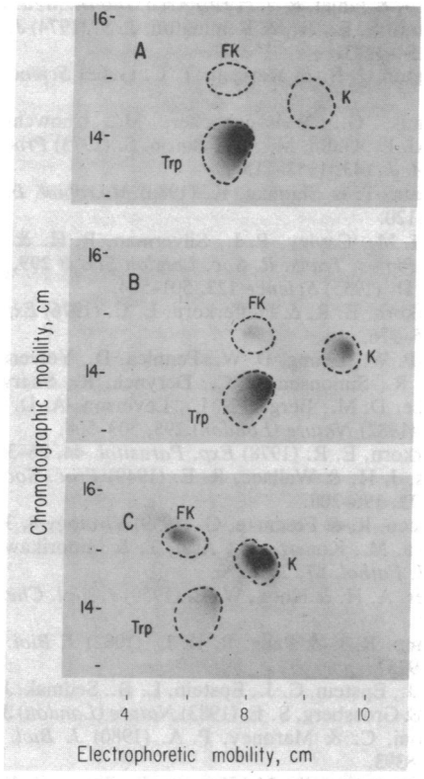

FIG. 5. Identification of the principal degradation products of tryptophan that were released into the medium by cells treated with IFN-γ. Confluent human fibroblast cultures were incubated in medium that contained 64 units of IFN-γ per ml or in control medium. Both media contained 10 μCi of [^{14}C]tryptophan per ml. Samples of the medium were diluted 2-fold with an aqueous solution of tryptophan and kynurenine at 5 mg/ml each and *N*-formylkynurenine at 10 mg/ml. This mixture was analyzed by two-dimensional thin-layer chromatography and electrophoresis in a mixture of formic and acetic acids. The radioactive spots on the thin layer were located by autoradiography with an 8-day exposure. These autoradiograms contain all of the radioactive spots that were detected. The ninhydrin spots of tryptophan (Trp), kynurenine (K), and *N*-formylkynurenine (FK) are drawn on the developed x-ray film. (*A*) Control culture after incubation for 1 day. (*B* and *C*). IFN-γ-treated cultures after incubation for 1 day (*B*) and for 2 days (*C*).

Once the principal tryptophan metabolites found in the medium were identified, it was possible to re-examine the nature of the high level of radioactivity found in the acid-soluble pool of human fibroblasts that had been treated with IFN-γ (Fig. 4). Two-dimensional chromatographic/electrophoretic analyses of the acid-soluble pool extracted 5 min after exposure to medium that contained [^{3}H]tryptophan showed that <1% of the intracellular radioactivity was in the form of tryptophan, while kynurenine and *N*-formylkynurenine accounted for 30% and 70% respectively.

DISCUSSION

Treatment with IFN-γ induced human fibroblasts to degrade tryptophan. Although the enzymes involved in this degradation have not yet been measured, their identity seems clear on the basis of the principal tryptophan metabolites isolated from the medium. The main degradation product of radioactive tryptophan was proven to be kynurenine by cochromatography and coelectrophoresis with the authentic substance under a wide variety of conditions. This identification was confirmed by a comparison of fluorescence emission spectra. The second most prominent degradation product, *N*-formylkynurenine was identified only electrophoretically and chromatographically. However, the fact that *N*-formylkynurenine is known to be the biochemical intermediate between tryptophan and kynurenine (Fig. 6) serves to confirm its identification. Thus IFN-γ must, at a minimum, induce an increase in the activity of the enzyme tryptophan 2,3-dioxygenase (EC 1.13.11.11) that converts tryptophan to *N*-formylkynurenine. It remains to be determined if the activity of the second enzyme in the tryptophan degradation pathway, formamidase (aryl-formylamine amidohydrolase, EC 3.5.1.9) is also increased. It is certainly possible that both of these enzymes are induced because IFN-γ has been reported to elicit the synthesis of at least three new mRNAs (16) and six new proteins (17) in addition to those that were induced also by IFN-α and IFN-β.

The role of tryptophan degradation in the antitoxoplasma state induced by IFN-γ should first be considered in the context of what is known of the antiviral activities of interferon. Work in this laboratory has shown that the antitoxoplasma effect of IFN-γ resembles the antiviral action of interferon in at least three ways (unpublished data). (*i*) IFN-γ has no effect on extracellular parasites. (*ii*) The host cells must be treated with IFN-γ for $\approx$1 day before an antitoxoplasma state is established. (*iii*) Once the antitoxoplasma state is established, the medium in which the IFN-γ was supplied can be replaced with normal medium, and the growth of *T. gondii* is still blocked.

The biochemical bases of the antiviral state induced by the various interferons are now reasonably well understood (reviewed in ref. 7). Treatment with interferon induces the synthesis of two enzymes, (2′-5′)oligo(A) polymerase (designat-

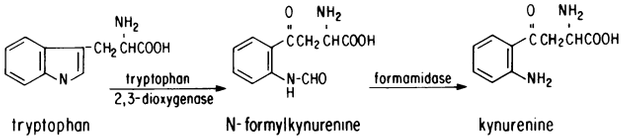

FIG. 6. The pathway from tryptophan to kynurenine as originally defined by Mehler and Knox (15).

ed (2'-5')A polymerase), and a protein kinase. Each of these enzymes, when activated by double-stranded RNA made during viral infection, serves to compromise protein synthesis. The (2'-5')A polymerase makes (2'-5')oligo(A), a series of short oligonucleotides in which the adenylic acid residues are linked by a (2'-5')phosphodiester bond. A (2'-5')A polymerase (18, 19) and a kinase (20) that phosphorylates initiation factor eIF-2 are also induced by IFN-γ, but their roles in the antiviral action of this interferon have not been conclusively defined.

The possible antitoxoplasma role of the host cell (2'-5')A polymerase and protein kinase induced by IFN-γ must be considered with reference to the intracellular site of *T. gondii*. This parasite grows within a membrane-bound structure, the parasitophorous vacuole, that lies within the cytoplasm of its host cell (14). Thus, two barriers that are probably impermeable to proteins, the vacuolar membrane and the plasma membrane of the parasite itself, are interposed between the cytoplasm of the host cell and that of *T. gondii*. It is unlikely that the enzymes induced by interferon have access to intracellular *T. gondii*. Similarly, the effector molecule (2'-5')oligo(A) would not be expected to cross membranes. *T. gondii* is so well insulated from the macromolecules of its host cell that the antitoxoplasma state induced by IFN-γ is likely to be mediated by substances of low molecular weight. Further direct evidence that the antiviral proteins that are induced by all interferons cannot be involved in the antitoxoplasma activity of IFN-γ comes from the observation that only this interferon suppresses the growth of *T. gondii*. Both human recombinant IFN-α and IFN-β had no antitoxoplasma effect at 1,024 units/ml, the highest concentrations tested (unpublished data).

The observations reported here provide an alternative biochemical explanation for the antitoxoplasma state produced in human fibroblasts by IFN-γ. As discussed above, treatment with IFN-γ induced the degradation of tryptophan into kynurenine, *N*-formylkynurenine, and several unidentified products. It is most unlikely that the interferon-induced degradation of tryptophan and the suppression of the growth of *T. gondii* are unrelated events. Any hypotheses that link these two events must explain the observation that small increases in tryptophan concentration can block the antitoxoplasma activity of a given concentration of IFN-γ (Fig. 2). In constructing these hypotheses, the assumption was made that *T. gondii*, like its host cell, was incapable of tryptophan synthesis.

The intracellular degradation of tryptophan by the host cell in response to treatment with IFN-γ could restrict the growth of *T. gondii* in at least two ways. One or more of the tryptophan metabolites could be toxic to the parasite in a manner that was reversible by tryptophan. Alternatively, the degradation of tryptophan could so reduce its concentration in the acid-soluble pool of the host cell that the intracellular parasites would be starved for an essential amino acid. The first of these hypotheses is readily testable by examining the effect of ultrafiltrates of medium from IFN-γ-treated cultures. The low amounts of tryptophan in the acid-soluble pool of cultures treated with IFN-γ provide suggestive evidence for the second hypothesis.

Both crude and purified natural human IFN-γ also induce fibroblasts to degrade tryptophan (unpublished data). However, it remains to be determined if the antitoxoplasma state produced *in vitro* by the degradation of tryptophan has any significance *in vivo*, where the concentration of tryptophan in blood is about 7 μg/ml (21). It should be noted that there is no evidence that infection with *T. gondii* was needed to trigger the IFN-γ-treated cells to degrade tryptophan. The experiments that described this phenomenon were carried out with uninfected cells. The antiproliferative effect of IFN-γ on cultured cells and its toxic effects *in vivo* may also be a consequence of tryptophan degradation and, thus, may be reversible by an increased supply of this amino acid.

Note Added in Proof. When this manuscript was prepared, I was unaware of the important observation that high doses of IFN-β (10^4 units/ml) induced indoleamine 2,3-dioxygenase [indoleamine:oxygen 2,3-oxidoreductase (decyclizing)] in mouse lung slices (22). I am indebted to Dr. Carl Nathan for bringing this work to my attention. The enzyme induced by physiological doses of INF-γ described in this report is probably also indoleamine dioxygenase.

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